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Colocalization of GLUT2 Glucose Transporter, Sodium/Glucose Cotransporter, and γ-Glutamyl Transpeptidase in Rat Kidney With Double-Peroxidase Immunocytochemistry

STEVEN C. CRAMER, WILLIAM M. PARDRIDGE, BRUCE A. HIRAYAMA, AND ERNEST M. WRIGHT

Glucose is reabsorbed from the glomerular filtrate in the proximal segment of the renal tubule in two stages. The first stage is uphill transport across the brush border membrane by Na^+-glucose cotransport and the second stage is downhill transport across the basolateral membrane by facilitated diffusion. Genes for both a renal Na^+-glucose cotransporter (SGLT1) and a renal facilitated glucose transporter (GLUT2) have been cloned and sequenced. To examine whether SGLT1 and GLUT2 colocalize to the same tubular epithelial cells in rat kidney, double-immunoperoxidase studies with dual chromogens and paraformaldehyde perfusion-fixed frozen sections of rat kidney were performed. Antipeptide antisera were prepared against rat GLUT2 (amino acids 510–522) and rabbit SGLT1 (amino acids 402–420). Proximal tubules were identified immunocytochemically with an antiserum raised against a synthetic peptide corresponding to the 21 amino acids at the COOH-terminal of the heavy chain of rat γ-glutamyl transpeptidase, which is a proximal tubule–specific enzyme. The anti-GLUT2 antiserum strongly stained the basolateral membrane of 46% of cortical tubules, whereas the SGLT1 antiserum stained the brush border of 56% of the cortical tubules. The γ-glutamyl transpeptidase antiserum also stained the brush border of 51% of the cortical tubules. GLUT2 and SGLT1 colocalized to 40% of cortical epithelium, but 16% of cortical epithelial cells were immunopositive for brush border SGLT1 and immunonegative for basolateral GLUT2. These γ-glutamyl transpeptidase staining results suggest that at least 50% of the tubules in the cortex are proximal tubules and that SGLT1 and GLUT2 colocalize to most proximal tubules. The fact that SGLT1 antiserum immunoreacted with tubules unreactive to the GLUT2 antiserum suggests that either the SGLT1 epitope is conserved on a related brush border protein or that there is another GLUT transporter responsible for the exit of sugar from these proximal tubule cells. *Diabetes* 41:766–70, 1992

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Glucose is >90% reabsorbed at the proximal tubule of the kidney (1,2). Physiological studies supported the model of glucose transport through two membranes in series, i.e., the brush border and basolateral membranes of the kidney tubule epithelium via the concerted action of a Na^+-glucose transporter and a Na^+-independent glucose transporter, respectively (3,4). The GLUT2 isoform of the Na^+-independent glucose transporter gene family has been localized to the most proximal (S1) portion of the proximal convoluted tubule with minimal GLUT2 immunostaining in S2 or S3 of the proximal convoluted tubule (5). The GLUT1 isoform (6) was also found in the basolateral membrane of kidney epithelium but was principally located in collecting duct epithelium (5,7,8). With regard to the Na^+-glucose cotransporter (SGLT1; 9–11), recent immunofluorescence studies localized this protein to the brush border membrane of S1, S2, and S3 of the rat proximal tubule (7).

Thus far, the GLUT2 and SGLT1 isoforms have not been colocalized to the same cells of kidney tubular epithelium. In this study, we examined the distribution of GLUT2 and SGLT1 transporters in rat kidney with immunocytochemical techniques, and the results provide direct information about the role of these transporters in glucose absorption across the proximal tubule.

RESEARCH DESIGN AND METHODS

Antiserum production. Synthetic peptides were prepared at the University of California at Los Angeles Peptide Synthesis Core Facility corresponding to the 13

amino acids (residues 510–522) at the COOH-terminal of rat GLUT2 (12) and the 21 amino acids of the COOH-terminal of rat γ-glutamyl transpeptidase (γ-GTP) heavy chain (13). The identity of the synthetic peptide was confirmed by amino acid composition analysis. The synthetic peptides were separately coupled to beef thyroglobulin with glutaraldehyde, and polyclonal antisera in rabbits were prepared as described previously (14). The GLUT2 antiserum was initially characterized with peroxidase immunocytochemistry in rat pancreas, and the γ-GTP heavy chain antiserum was initially characterized with Western-blot analysis (see below). The GLUT2 and γ-GTP antisera that were prepared with beef thyroglobulin as a carrier protein were preabsorbed with beef thyroglobulin before all immunocytochemical studies. This preabsorption was performed as follows: 10 mg beef thyroglobulin was added to 1 ml 1:100 antiserum dilution dissolved in 0.01 M Tris (pH 7.4)/0.15 M NaCl followed by incubation at 37°C for 60 min with shaking and centrifugation at 10,000 × *g* for 30 min at room temperature. The supernatant was transferred to a new tube, and the thyroglobulin absorption was twice repeated. For absorptions involving synthetic peptide, the same protocol was used except 1 mg synthetic peptide was used in lieu of 10 mg beef thyroglobulin. The preparation and characterization of the rabbit polyclonal antiserum prepared against the extracellular portion of SGLT1 (residues 402–420) has been previously described (15).

Antibody characterization. The specificity of the γ-GTP antiserum was assessed by immunoblotting (16) beef kidney γ-GTP; 3 μg purified γ-GTP was electrophoresed through a 12% sodium dodecyl sulfate–polyacrylamide gel followed by blotting to Immobilon-P filters (Millipore, Bedford, MA) in 20% methanol. The filter was probed with the anti–γ-GTP antiserum at a 1:100 dilution for 90 min at 37°C, and the bound antiserum was detected with an avidin-biotin peroxidase assay (14). Molecular-weight standards from Bio-Rad (Richmond, CA) were run in a parallel lane and stained with Coomassie Blue. The antiserum stained a single 64,000-M_r band corresponding to the expected size of the γ-GTP heavy chain (17). In the kidney, this brush border enzyme is preferentially localized in the S2 and S3 segments of the proximal tubule (18).

The GLUT2 antiserum stained the plasma membrane of β-cells of rat pancreatic islets with no staining of the peripheral α-cells (Fig. 1*A*). GLUT2 immunostaining of rat pancreatic islets was inhibited >75% in rats with streptozocin-induced experimental diabetes. The serum also stained sinusoidal plasma membranes of rat hepatocytes and the basolateral membrane of the rat intestinal epithelium (data not shown). This is the expected distribution of GLUT2 (5,12). The SGLT1 antiserum was tested on rat renal brush border membranes by Western blotting. Specific immunoreactivity was observed with a 63,000-M_r protein, which is consistent with previous studies of rat intestinal brush borders (15).

Tissue fixation and embedding. Aldehyde fixation did not impair the immunoreactivity of any of the three different antisera used. However, parathin embedding eliminated SGLT1 immunoreactivity and diminished γ-GTP immunostaining. Therefore, frozen sections were used. Adult male Sprague-Dawley rats were anesthetized with ketamine and xylazine and, after initial saline perfusion, were perfusion fixed with 2% paraformaldehyde via intracardiac perfusion. After perfusion fixation, slabs of kidney were immersed in 20% sucrose overnight at 4°C followed by the preparation of 10-μ frozen sections with a Bright cryostat at −20°C. In other studies, tissues were parathin embedded after perfusion or immersion fixation, and 6-μ sections were cut on a microtome.

Double immunostaining. Double-peroxidase immunocytochemistry was performed with the avidin-biotin peroxidase method (19–20) according to manufacturer's instructions (ABC Vectastain method) and used either 3-amino-9-ethylcarbazole (AEC), 3,3′-diamino-benzidene (DAB), or 4-chloro-1-naphthol (CN) as the final chromogen. In studies involving colocalization of GLUT2 and γ-GTP, the GLUT2/DAB system was used before the γ-GTP/AEC system because the use of the reverse order inhibited staining at the second step. Staining with DAB did not include the addition of nickel chloride in the final chromogen development step. The antisera were used at dilutions ranging from 1:250 to 1:750, and the primary antibody incubations ranged from 60 min at room temperature to overnight at 4°C. Immunostaining also was performed with either preimmune serum or synthetic peptide-absorbed antiserum.

The avidin-biotin immunoperoxidase reagents (ABC Vectastain) were obtained from Vector (Burlingame, CA). The AEC, DAB, CN, beef thyroglobulin, beef kidney γ-GTP, and all other reagents were purchased from Sigma (St. Louis, MO).

RESULTS

The GLUT2 antiserum stained the basolateral membranes of the rat renal cortical tubules (Fig. 1*B*). In colocalization experiments (Fig. 1*C* and *D*), the tubules that stained with GLUT2 also stained on the brush border membrane with the SGLT1 antiserum. The GLUT2 antibody did not react with the brush border membrane, and the SGLT1 antibody did not react with the basolateral membrane. At the level of the light microscope, there was no obvious staining of intracellular proteins or organelles with either antibody. Not all the SGLT1 immunoreactive tubules were costained with the GLUT2 antiserum (Fig. 1*D*). In two different inspections of 500 cortical tubules, 56% of the tubules were immunopositive for SGLT1, 47% were immunopositive for GLUT2, 40% were simultaneously immunopositive for both SGLT1 and GLUT2, and 16% were immunopositive for SGLT1 but were immunonegative for GLUT2. Only 7% were immunopositive for GLUT2 and immunonegative for SGLT1 (Table 1).

In parathin sections (Fig. 1*E* and *F*), inspection of 400 tubules demonstrated that 46% of the tubules were immunopositive for γ-GTP and 34% were immunopositive for GLUT2; 30% of the tubules were immunopositive for γ-GTP but immunonegative for GLUT2, and γ-GTP and GLUT2 colocalized in 16% of the tubules (Table 1).

We also attempted to colocalize γ-GTP and SGLT1

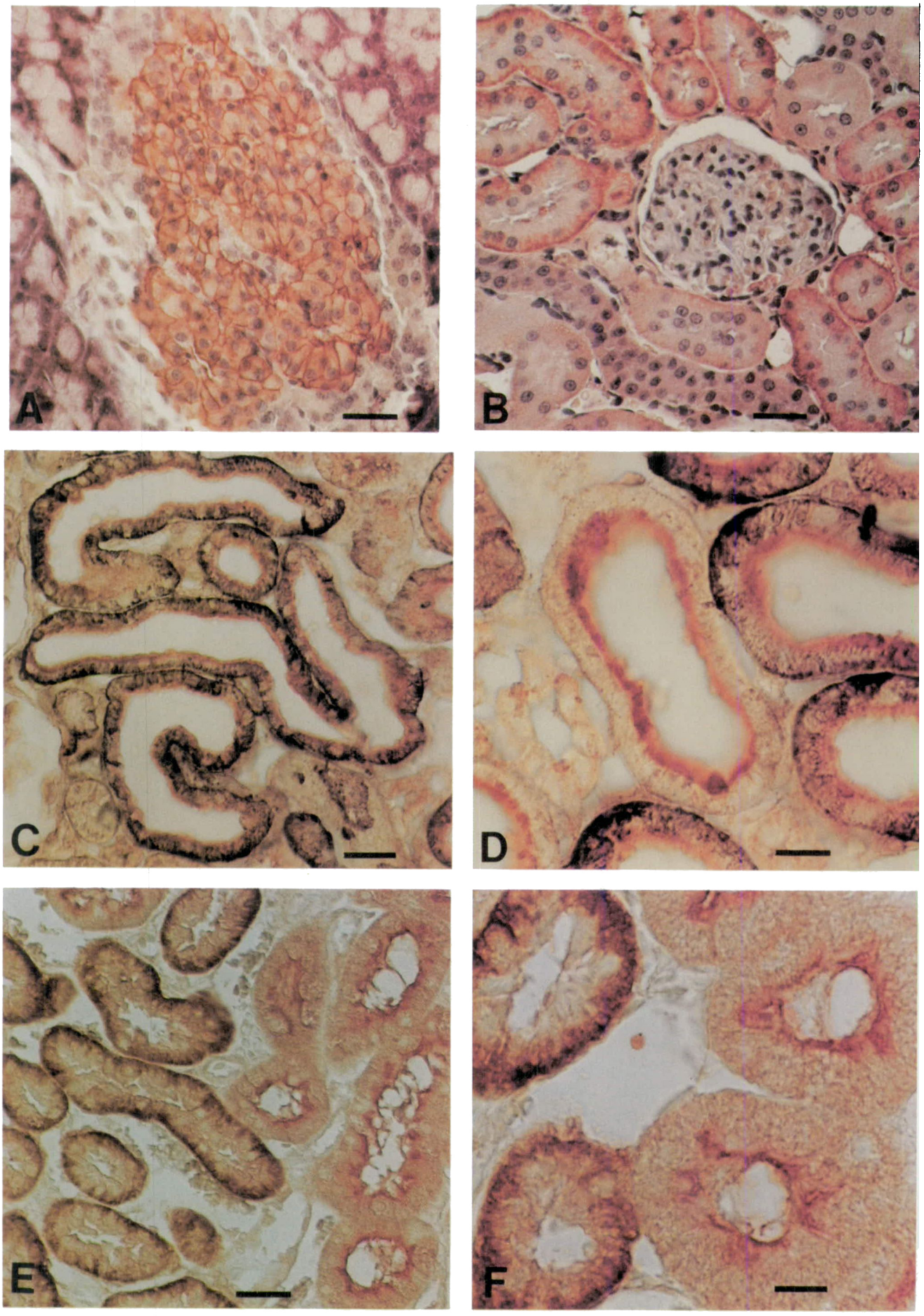
A
B
C
D
E
F

FIG. 1. *A*: paraformaldehyde immersion-fixed 6-μ parathin sections of rat pancreatic islets were immunostained with a 1:500 dilution of the anti-GLUT2 rabbit polyclonal antiserum and counterstained with Mayer's hematoxylin. *B*: paraformaldehyde immersion-fixed 6-μ parathin sections of rat kidney were immunostained with a 1:250 dilution of GLUT2 antiserum and counterstained with Mayer's hematoxylin. *C* and *D*: after paraformaldehyde perfusion fixation, sucrose infiltration, and preparation of 10-μ cryostat sections, rat kidney was sequentially immunostained with the GLUT2 antiserum and a chloronaphthol chromogen (*black color*) followed by labeling with the sodium/glucose cotransporter antiserum and staining with the 3-amino-9-ethylcarbazole chromogen (*red color*). *E* and *F*: paraformaldehyde immersion-fixed 6-μ parathin sections of control rat kidney were double-immunostained with the GLUT2 antiserum and a diaminobenzidene (without nickel chloride) chromogen (*brown color*) and the γ-GTP antiserum with 3-amino-9-ethylcarbazole as the chromogen (*red color*). Sections in *C*, *D*, *E*, and *F* were not counterstained. *Bar*, 28 μ (*A,B,C,E*) and 11 μ (*D,F*).

immunoreactivity on the same sections. Because both antibodies react with brush border membranes, it was difficult to obtain quantitative estimates of the colocalization. Qualitatively, we estimate that these antibodies colocalize to the brush borders of the same tubules.

No specific immunostaining was observed with either preimmune sera or antisera preabsorbed with the respective synthetic peptide.

DISCUSSION

In this study, we used double-peroxidase staining procedures to colocalize the immunoreactive GLUT2 and SGLT1 glucose transport proteins. To facilitate the identification of proximal tubules in sections of the rat renal cortex, we used an antibody to the brush border marker γ-GTP. In the kidney, this brush border enzyme is preferentially localized in the S2 and S3 segments of the proximal tubule (18). The observation that γ-GTP and GLUT2 generally did not colocalize (Table 1) suggests a predominant S1 localization of GLUT2, which has been previously observed by Thorens et al. (5). With γ-GTP as a proximal tubule marker, our immunocytochemical findings indicate >50% of the cortical tubules are proximal tubules. Our double-staining procedure indicates that SGLT1 is found in the brush border membrane of the γ-GTP immunopositive tubules. The GLUT2 antibody stained the basolateral membrane of most tubules that were SGLT1 immunopositive. This suggests that in most proximal tubules SGLT1 and GLUT2 are responsible for the reabsorption of glucose from the glomerular filtrate. These colocalization experiments confirm and extend the observation of Thorens et al. (5) and Takata et al. (7) on the distribution of GLUT2 and SGLT1 in rat kidney cortex.

The colocalization of GLUT2 and SGLT1 to the S1 segment of the proximal renal tubule in the rat is of interest because the Na^+-glucose co-transporter in this segment transports glucose with low affinity (1–4). However, the cloned SGLT1 transports glucose with high affinity after expression in frog oocytes (21). The antiserum to SGLT1 used in these studies recognizes a high-affinity Na^+-glucose cotransporter in rabbit kidney (21). Therefore, these data are consistent with two explanations. First, an SGLT1-related protein may mediate low-affinity glucose reabsorption in the S1 portion of the proximal tubule and this protein may be recognized by the antiserum used in these studies. Second, SGLT1 may function as a low-affinity glucose-Na^+ cotransporter in the early segments of the proximal tubule in the rat.

Our observation that 16% of the tubules were immunoreactive for SGLT1 but not for GLUT2 (Fig. 1*D*) indicates that either another GLUT transporter accounts for the final stage of glucose reabsorption in these tubules or that the SGLT1 antibody recognizes the same epitope in another brush border protein. Previous studies (7) did not find GLUT4 in proximal tubules, and recent work in this laboratory did not find GLUT3 (22) in formalin-fixed parathin-embedded sections of human autopsy kidney (unpublished observations). However, GLUT1 is present in the S3 segment of cortical tubules (5).

In summary, these studies describe methods for colocalization of sodium-dependent and sodium-independent transporters in kidney with immunoperoxidase histochemistry. These methods use light microscopy and allow for a semiquantitative analysis of glucose transporter abundance and localization. A precise identification of changes in transporter abundance with immunocytochemistry is limited by the heterogenous immunostaining of different tubules. Nevertheless, the alterations in brush border SGLT1 and basolateral GLUT2 transporters in kidney epithelium in pathological states such as diabetes mellitus may be examined qualitatively in future studies.

TABLE 1
Colocalization of immunoreactive SGLT-1, GLUT-2, or glutamyltranspeptidase (γ-GTP) in rat kidney cortex

Basolateral reactivity (%)	Brush border reactivity (%)			
	SGLT1 (−)	SGLT (+)	γ-GTP (−)	γ-GTP (+)
GLUT2 (−)	37 ± 5	16 ± 1	37 ± 2	30 ± 1
GLUT2 (+)	7 ± 2	40 ± 5	18 ± 2	16 ± 5

Data are means ± SD computed from counting 400–500 cortical tubules at 2 different examinations and represent the percentage of all cortical tubules counted that are immunoreactive, either immunopositive (+) or immunonegative (−), for GLUT2, SGLT1, or γGTP. SGLT, sodium/glucose cotransporter.

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REFERENCES

1. Tune B, Burg MB: Glucose transport by proximal renal tubules. *Am J Physiol* 221:580–85, 1971
2. Barfuss DW, Schafer JA: Differences in active and passive glucose transport along the proximal nephron. *Am J Physiol* 240:F322–32, 1981

3. Ullrich KJ: Renal tubular mechanisms of organic solute transport. *Kidney Int* 9:134–48, 1976
4. Silverman M: Glucose transport in the kidney. *Biochim Biophys Acta* 457:303–51, 1976
5. Thorens B, Lodish HF, Brown D: Differential localization of two glucose transporter isoforms in rat kidney. *Am J Physiol* 259:C286–94, 1990
6. Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, Allard WJ, Lienhard GE, Lodish HF: Sequence and structure of a human glucose transporter. *Science* 229:941–45, 1985
7. Takata K, Kasahara T, Kasahara M, Ezaki O, Hirano H: Localization of Na^+-dependent active type and erythrocyte/HepG2-type glucose transporters in rat kidney: immunofluourescence and immunogold study. *J Histochem Cytochem* 39:287–98, 1991
8. Farrell CL, Yang J, Pardridge WM: GLUT-1 glucose transporter is present within apical and basolateral membranes of brain epithelial interfaces and in microvascular endothelia barriers with and without tight junctions. *J Histochem Cytochem* 40:193–99, 1992
9. Coady MJ, Pajor AM, Wright EM: Sequence homologies among intestinal and renal Na^+/glucose cotransporter. *Am J Physiol* 259: C605–10, 1990
10. Morrison AI, Panayotova-Heirmann M, Feigl G, Scholermann B, Kinner RKH: Sequence comparison of the sodium–D-glucose cotransport systems in rabbit renal and intestinal epithelia. *Biochim Biophys Acta* 1089:121–23, 1991
11. Ohta T, Isselbacher KJ, Rhoads DB: Regulation of glucose transporters in GLC-PK cells: effects of D-glucose and monosaccharides. *Mol Cell Biol* 10:6491–99, 1990
12. Thorens B, Sarkar HK, Kaback HR, Lodish HF: Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney and β-pancreatic islet cells. *Cell* 55:281–90, 1988
13. Laperche Y, Bulle F, Aissani T, Chobert M-N, Aggerbeck M, Hanoune J, Guellaen G: Molecular cloning and nucleotide sequence of rat kidney γ-glutamyl transpeptidase cDNA. *Proc Natl Acad Sci USA* 83:937–41, 1986
14. Pardridge WM, Triguero D, Farrell CR: Downregulation of blood-brain barrier glucose transporter in experimental diabetes. *Diabetes* 39:1040–44, 1990
15. Hirayama BA, Wong HC, Smith CD, Hagenbuch BA, Hediger MA, Wright EM: Intestinal and renal Na^+/glucose cotransporters share common structures. *Am J Physiol* 261:C296–304, 1991
16. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76:4350–54, 1979
17. Tate SS, Khadse V, Wellner D: Renal γ-glutamyl transpeptidases: structural and immunological studies. *Arch Biochem Biophys* 262: 397–408, 1988
18. Rutenburg AM, Kim H, Fishbein JW, Hanker JS, Wasserkrug HL, Seligman AM: Histochemical and ultrastructural demonstration of γ-glutamyl transpeptidase activity. *J Histochem Cytochem* 17: 517–26 1969
19. Hsu S, Raine L, Fanger H: A comparative study of the peroxidase method and an avidin-biotin complex method for studying polypeptide hormone with radioimmunoassay antibodies. *J Clin Pathol* 75:734–38, 1981
20. Pardridge WM, Boado RJ, Farrell CR: Brain-type glucose transporter (GLUT-1) is selectively localized to the blood-brain barrier: studies with quantitative Western blotting and in situ hybridization. *J Biol Chem* 265:18035–40, 1990
21. Pajor AM, Hirayama BA, Wright EM: Molecular evidence for two renal Na+/glucose cotransporters. *Biochim Biophys Acta.* In press
22. Kayano T, Fukumoto H, Eddy RL, Fan Y-S, Byers MG, Shows TB, Bell GI: Evidence for a family of human glucose transporter-like proteins: sequence and gene localization of a protein expressed in fetal skeletal muscle and other tissues. *J Biol Chem* 263:15245–48, 1988